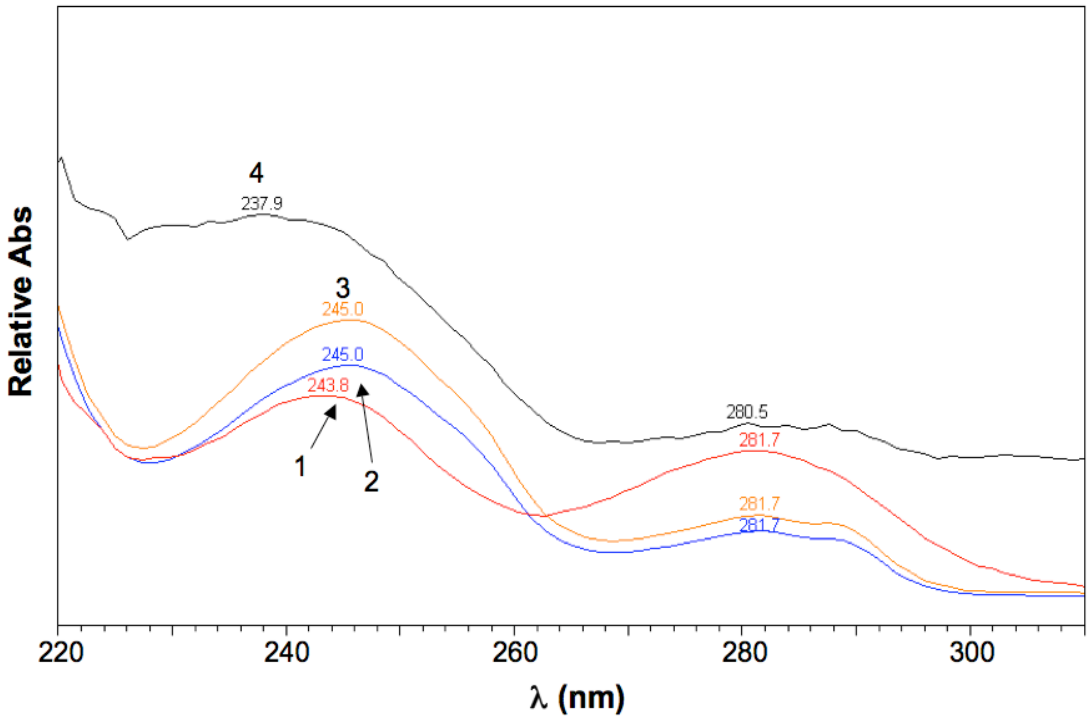
CHARACTERIZATION OF AN AM404 ANALOG, *N*-(3-HYDROXYPHENYL)-ARACHIDONOYLAMIDE, AS A SUBSTRATE AND INACTIVATOR OF PROSTAGLANDIN ENDOPEROXIDE SYNTHASE

Melissa V. Turman[‡,§], *Philip J. Kingsley*[‡], *and Lawrence J. Marnett*[‡,*]

[‡]From A. B. Hancock, Jr. Memorial Laboratory for Cancer Research, Departments of Biochemistry, Chemistry, and Pharmacology, Vanderbilt Institute of Chemical Biology, Center in Molecular Toxicology, and Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, Tennessee 37232 USA

SUPPORTING INFORMATION

A



B

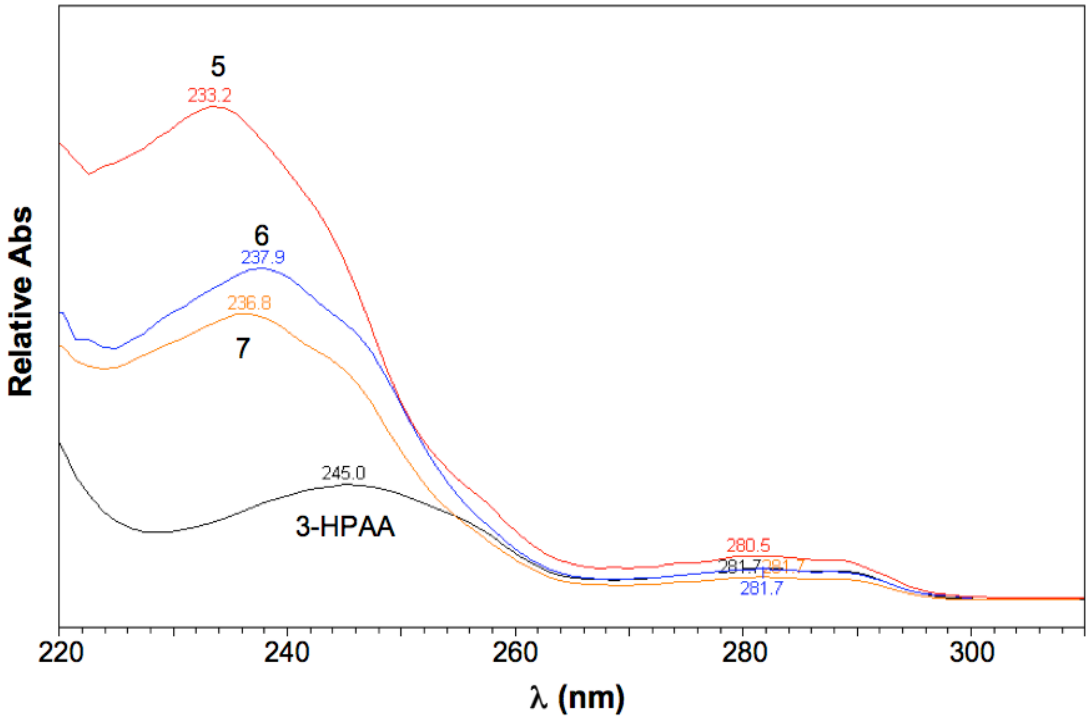


Figure S1. UV profiles of 3-HPAA and its products of oxygenation by PGHS-2. Labels indicate the product number assigned in Fig. 3 of the main text.

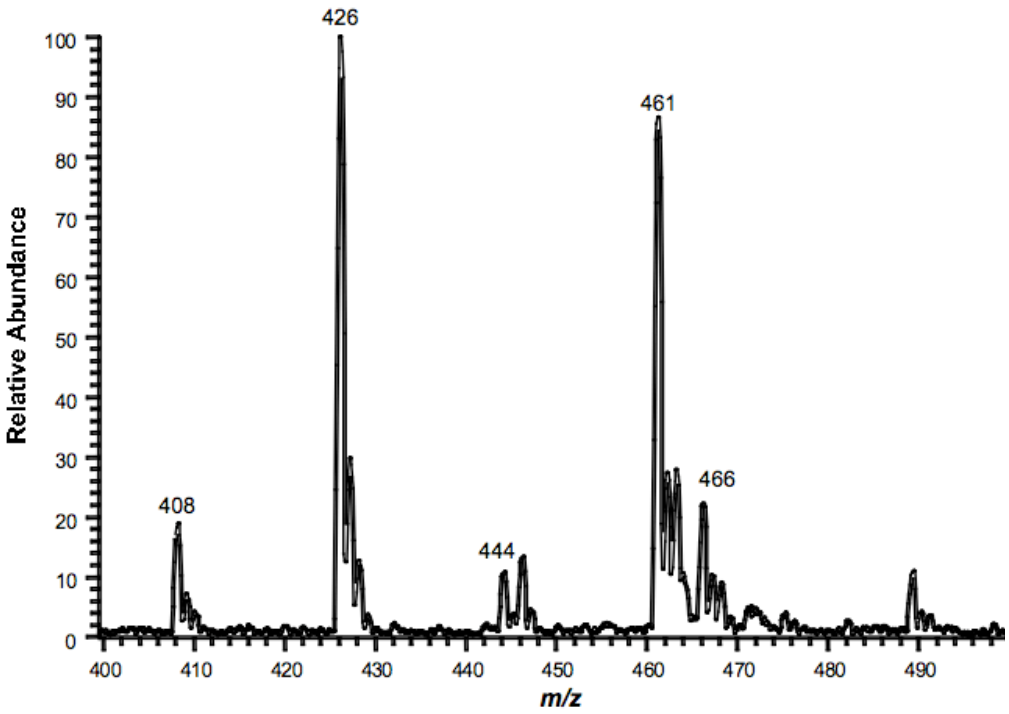
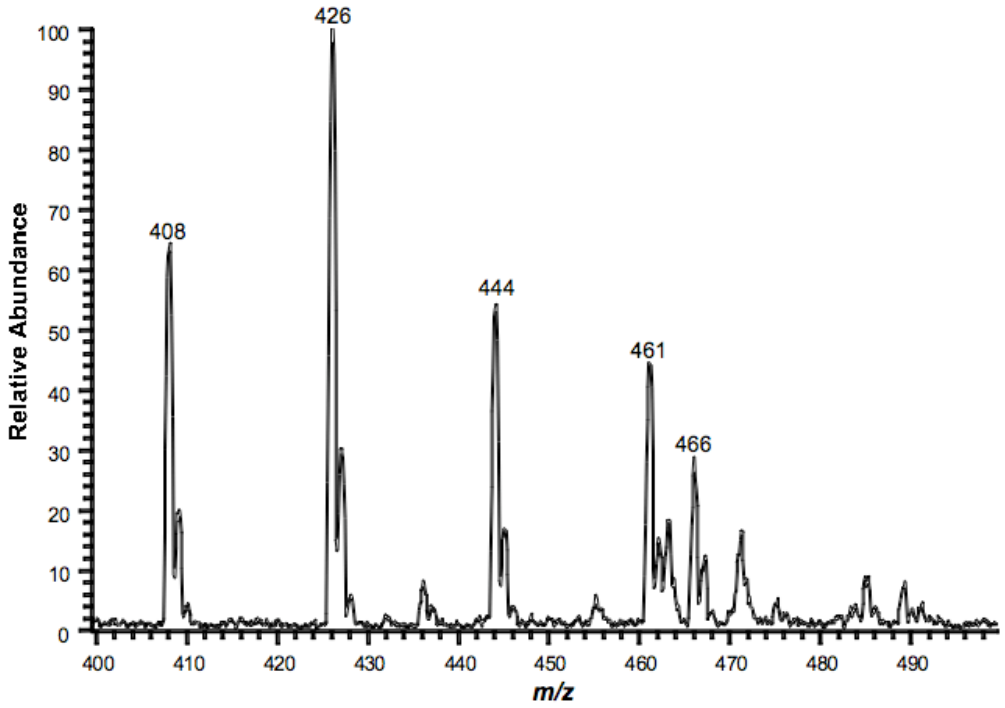


Figure S2. Full scan MS of product 3 formed by oxygenation of 3-HPAA by PGHS-2.

A



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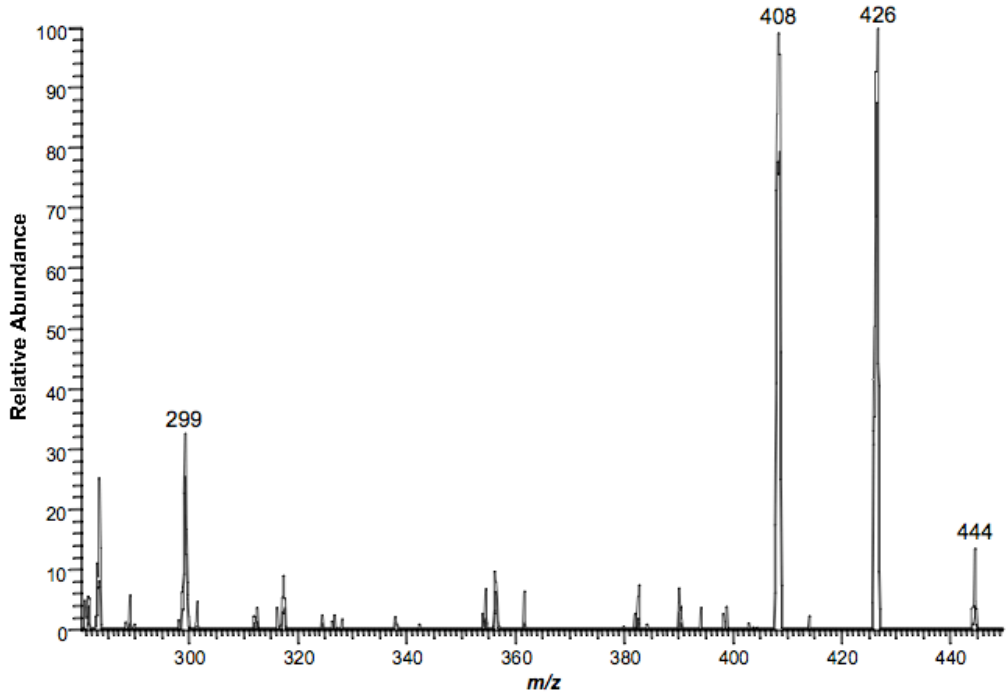


Figure S3. Mass spectral characterization of a prostaglandin-like product formed by oxygenation of 3-HPAA by PGHS-2. *A*, Full scan MS of peak 4. *B*, CID of *m/z* 444 at peak 4. Fragmentation is similar to that of the product at peak 2 (see Fig. 5, Results).

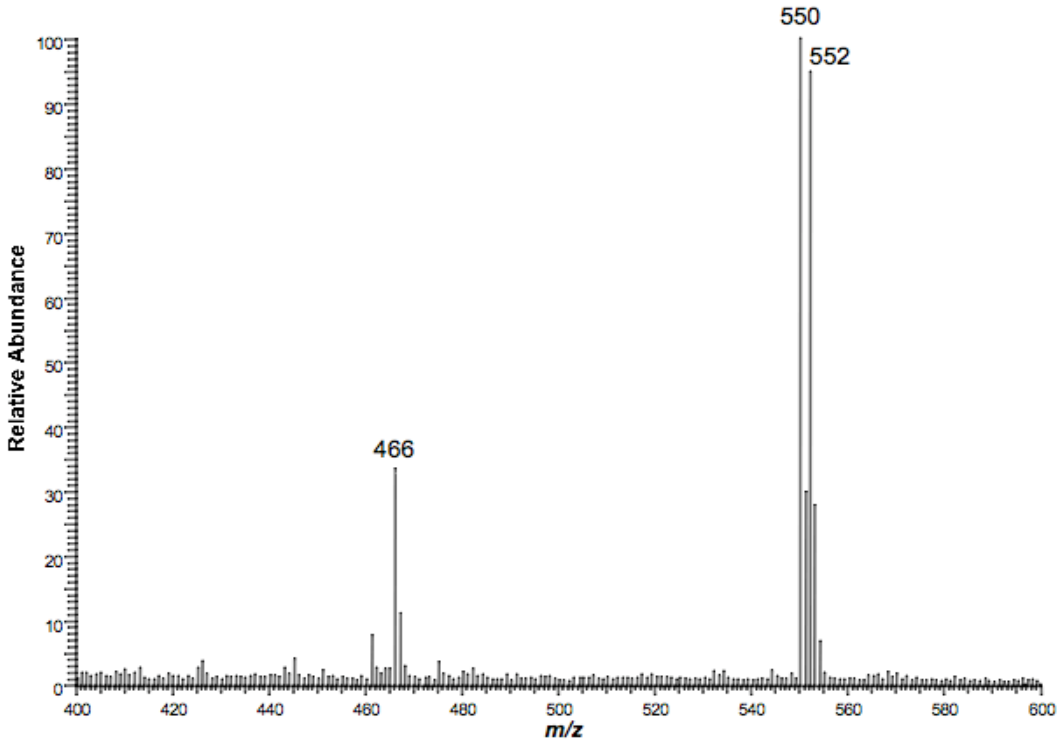
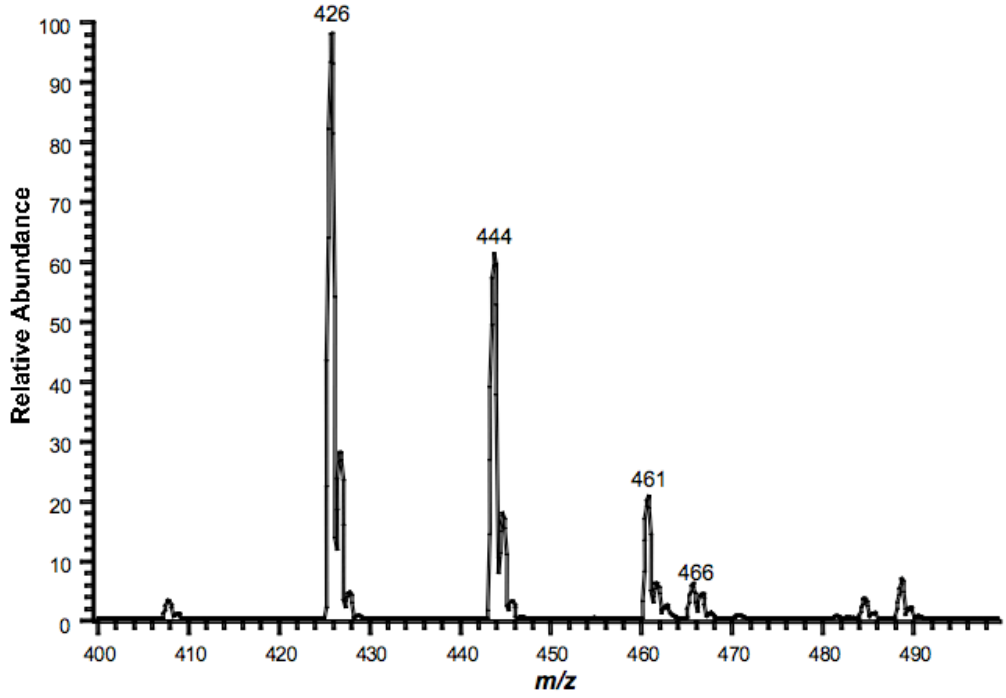


Figure S4. Full scan Ag^+CIS-MS spectrum of product 2. Ions with *m/z* of 550 and 552 correspond to a product with mass of 443 coordinated with $^{107}Ag^+$ and $^{109}Ag^+$. Ion with *m/z* of 466 corresponds to the sodiated adduct.

A



B

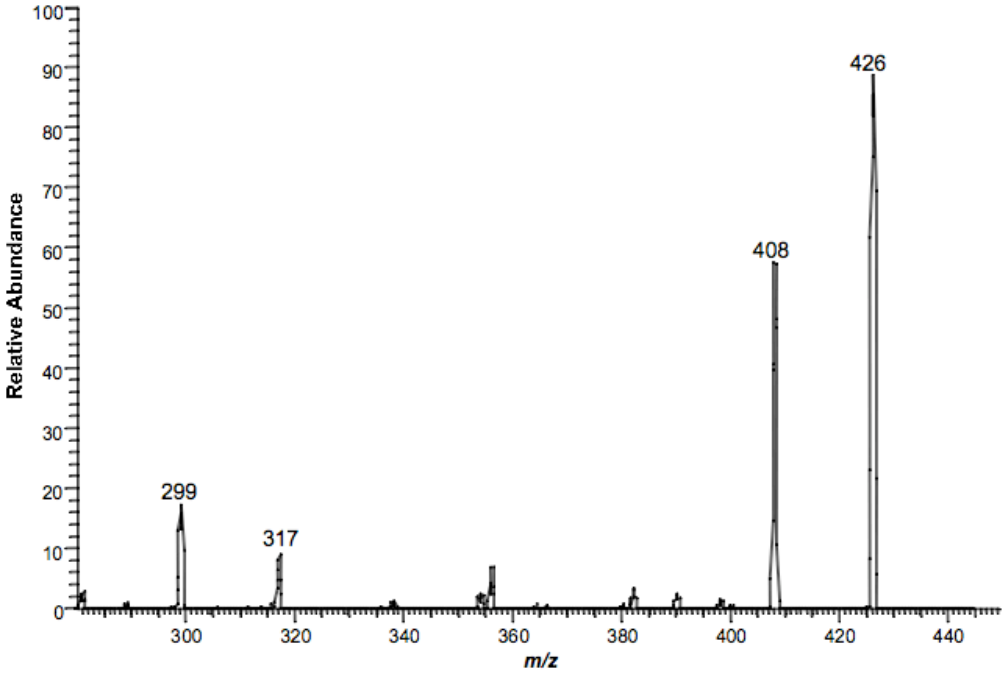
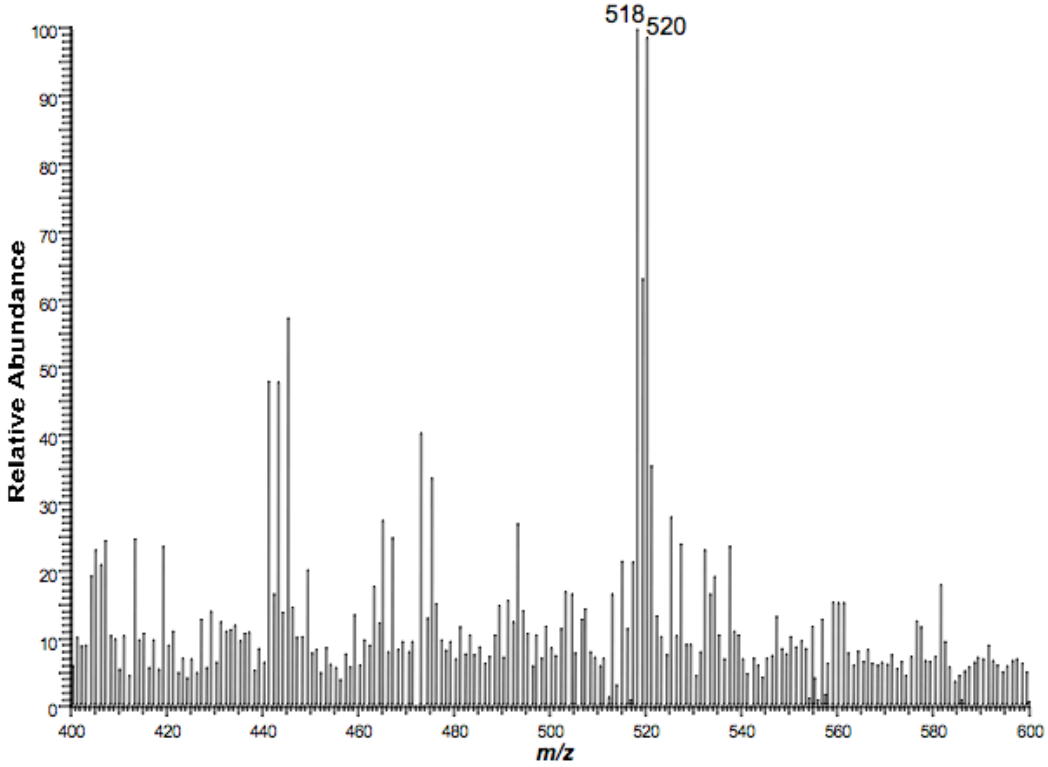
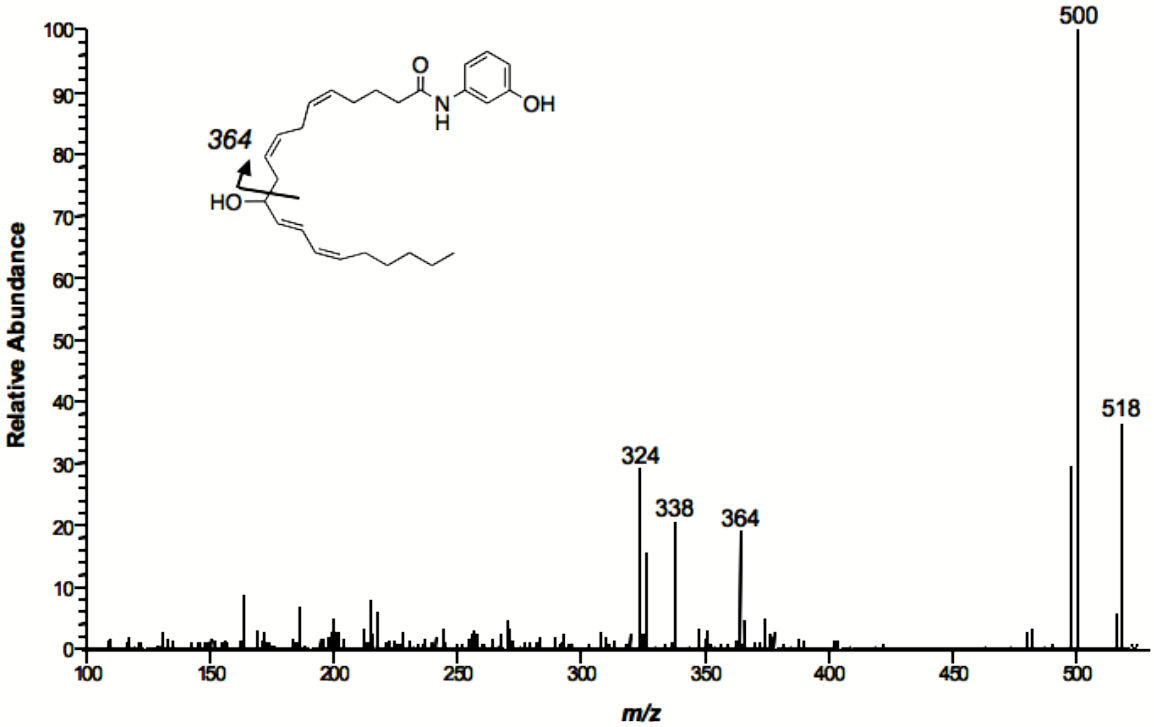


Figure S5. Mass spectral characterization of PGE_2-PA synthetic standard. *A*, Full scan MS. *B*, CID of *m/z* 444.

A


518
520
Relative Abundance
m/z

B


500
518
324
338
364
364
HO
O
N
H
OH
Relative Abundance
m/z

C

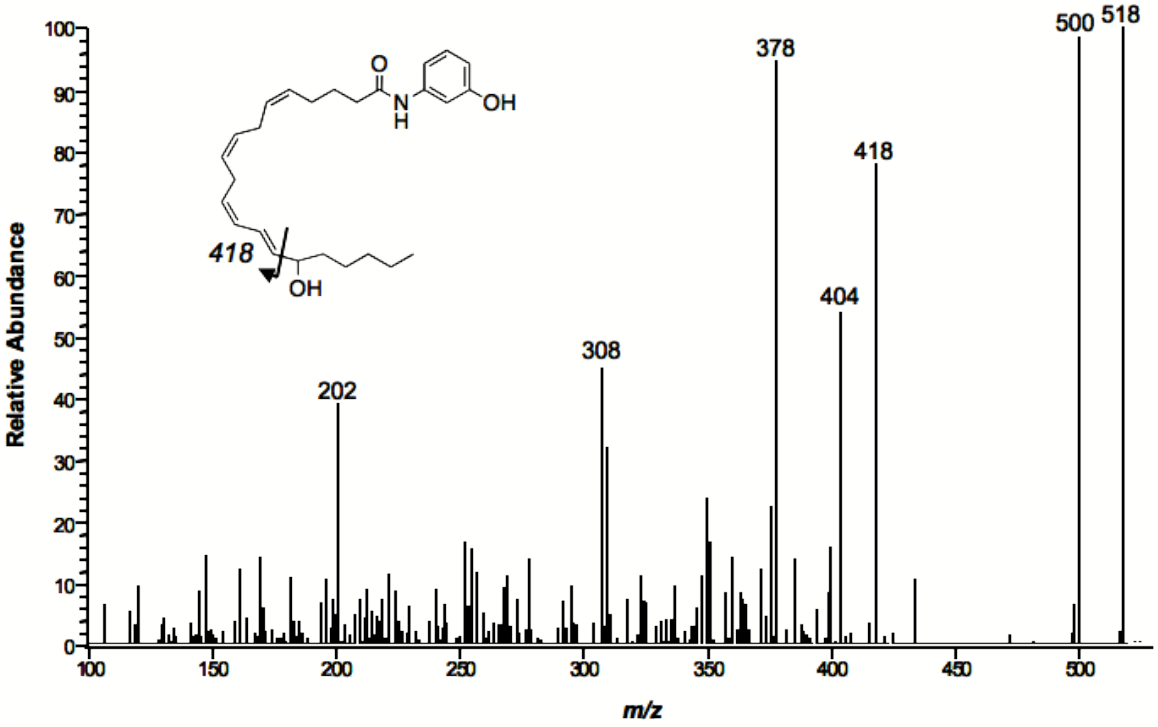
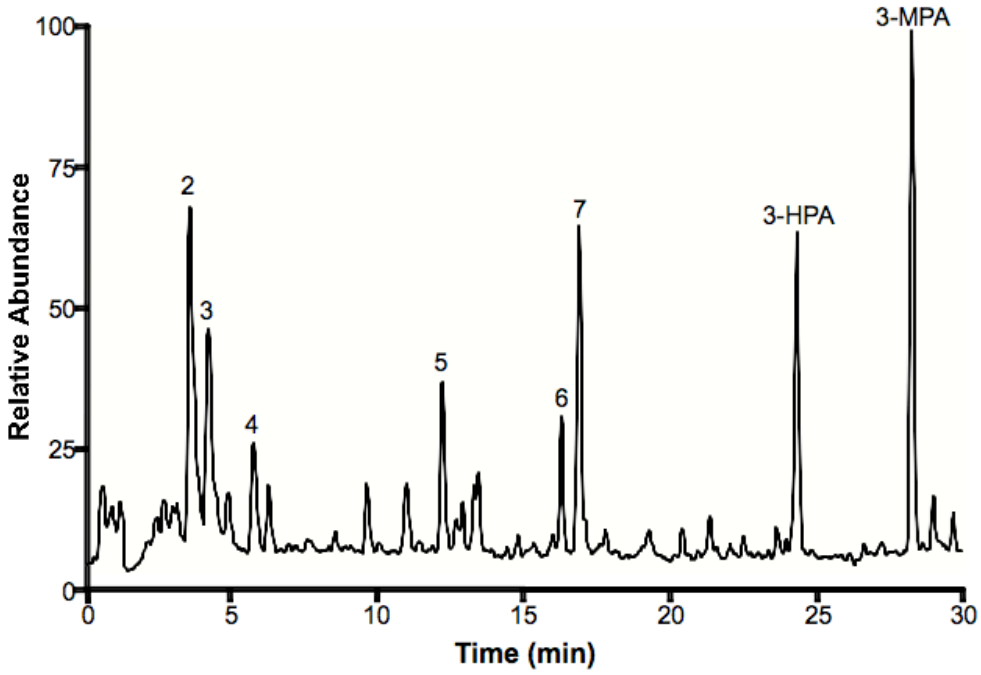


Figure S6. Ag^{+}CIS mass spectral characterization of HETE-like products formed following oxygenation of 3-HPAA by PGHS-2. *A*, Full scan MS of peak 7. Ions with *m/z* of 518 and 520 correspond to a product with mass of 411 coordinated with $^{107}Ag^{+}$ and $^{109}Ag^{+}$. A similar full scan MS was observed for peak 6. CID of *m/z* 518 yielded distinct spectra at *B,* peak 7 and *C,* peak 6. Both products exhibited an initial neutral loss of water (loss of 18 mu). Fragment ions with *m/z* of 364 (*B*) and 418 (*C*) are consistent with fragmentation α to the alcohol of 11-HETE-PA and 15-HETE-PA, respectively.

A



B

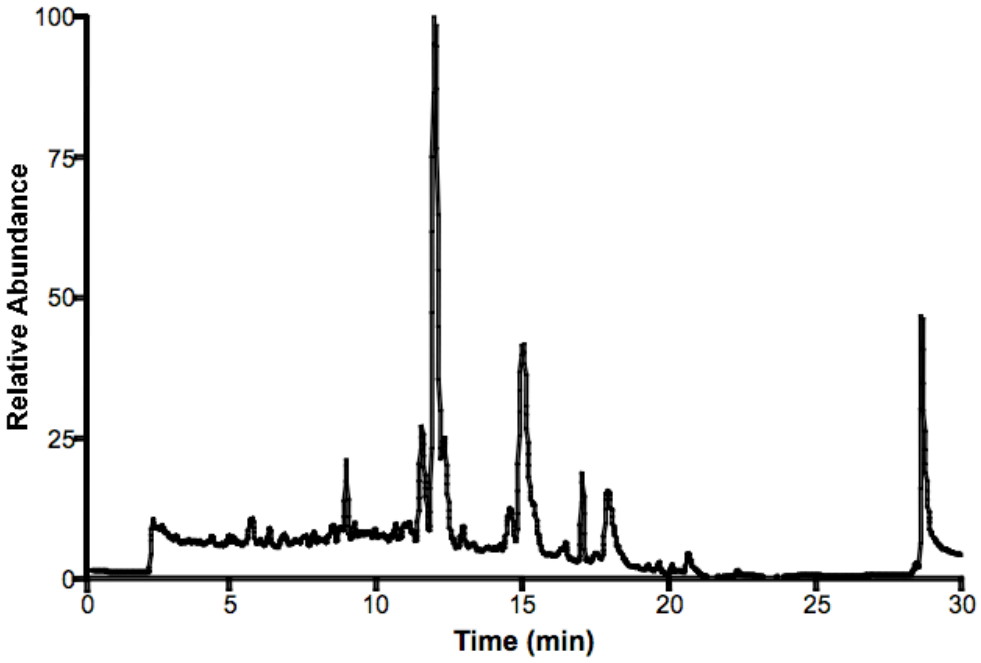


Figure S7. Total ion chromatograms for reactions of *A,* PGHS-2 and *B,* PGHS-1 with 3-HPAA.

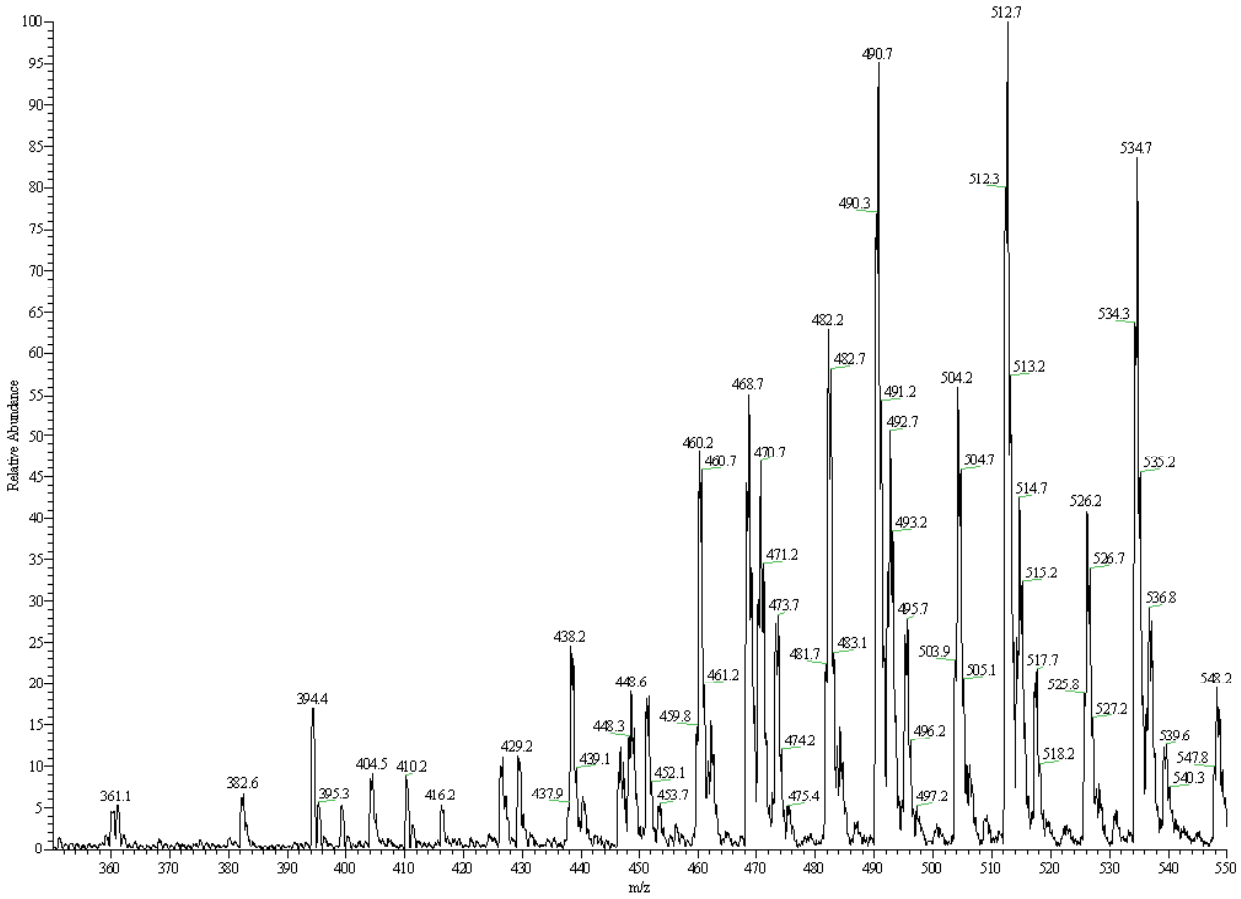
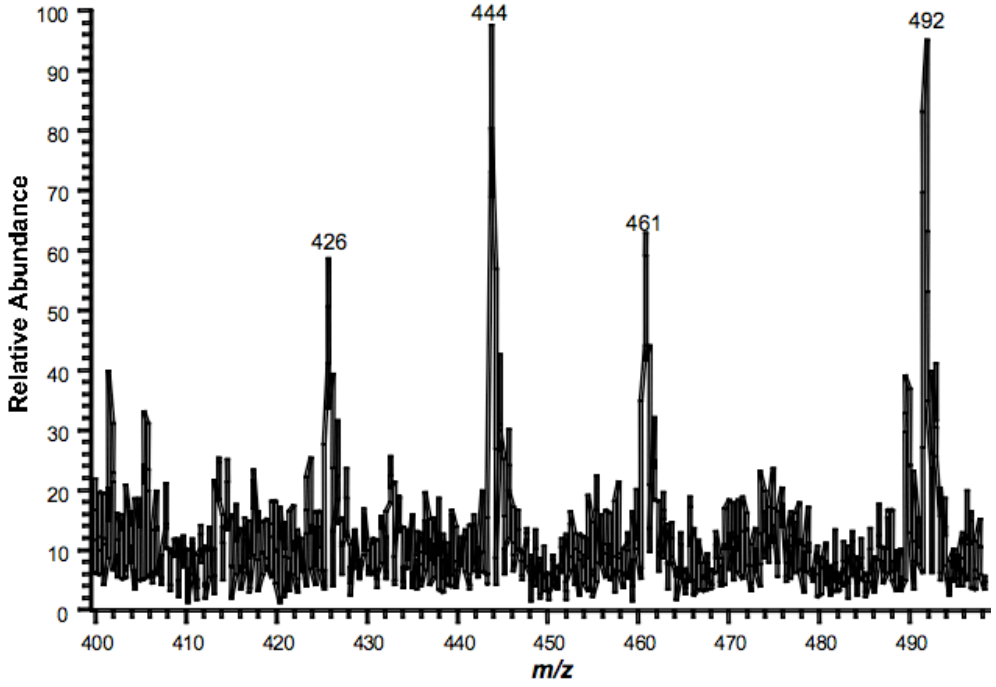


Figure S8. Full scan MS spectrum of peak observed at 12 min for PGHS-1 (See Fig. S6A).

A



B

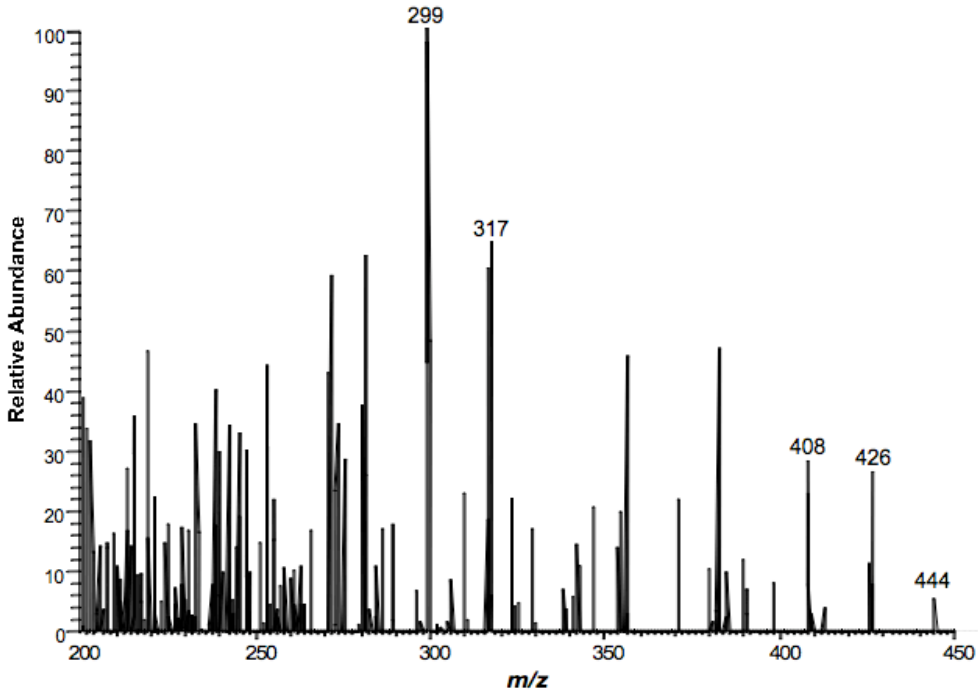


Figure S9. Mass spectral characterization of a prostaglandin-like product formed by oxygenation of 3-HPAA by PGHS-1. *A*, Full scan MS of product corresponding to peak 2 for PGHS-1. *B*, CID of *m/z* 444.

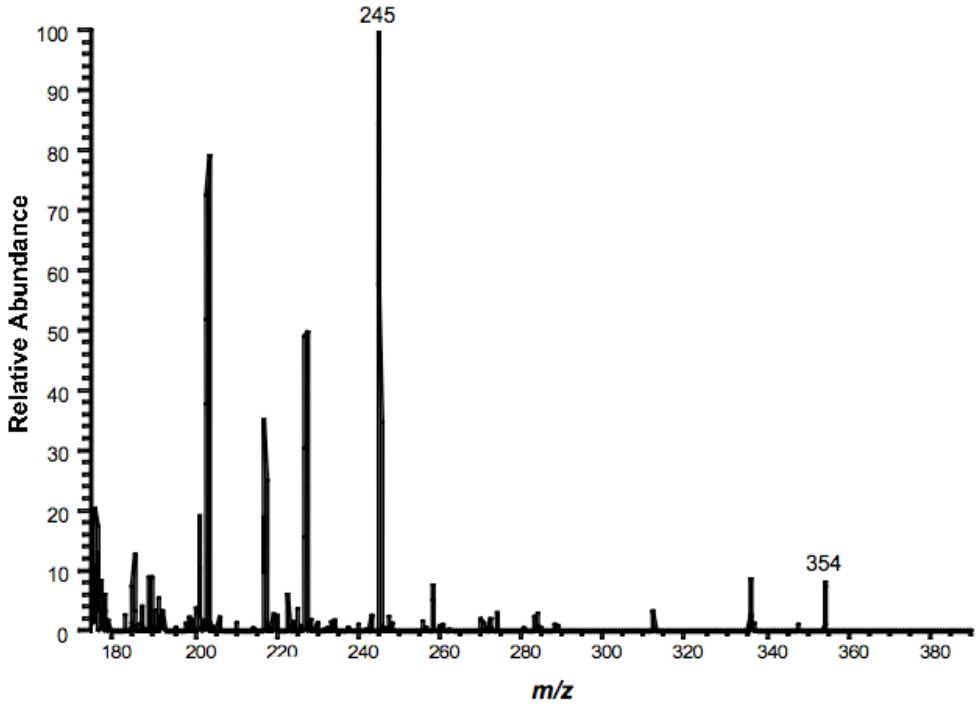
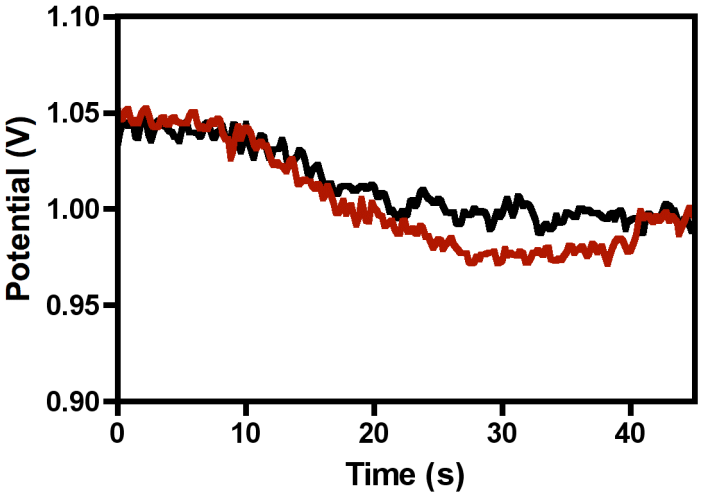


Figure S10. Mass spectral characterization of an HHT-like product formed by oxygenation of 3-HPAA by PGHS-1. CID of *m/z* 389 allowed observance of a product with similar retention time and fragmentation spectrum of product 5.

A



B

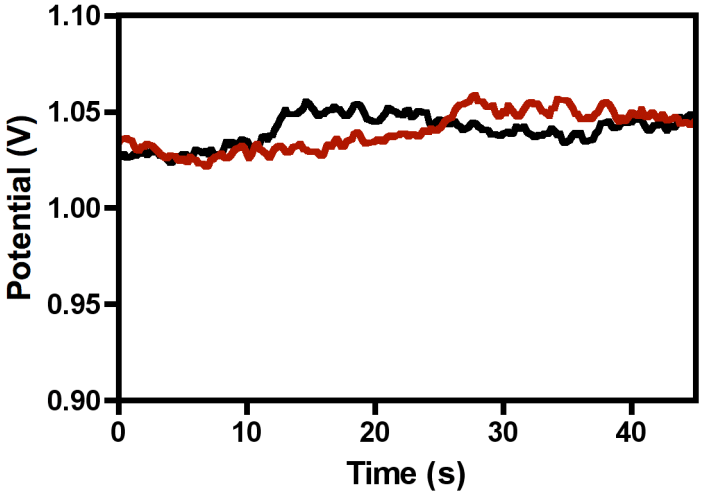
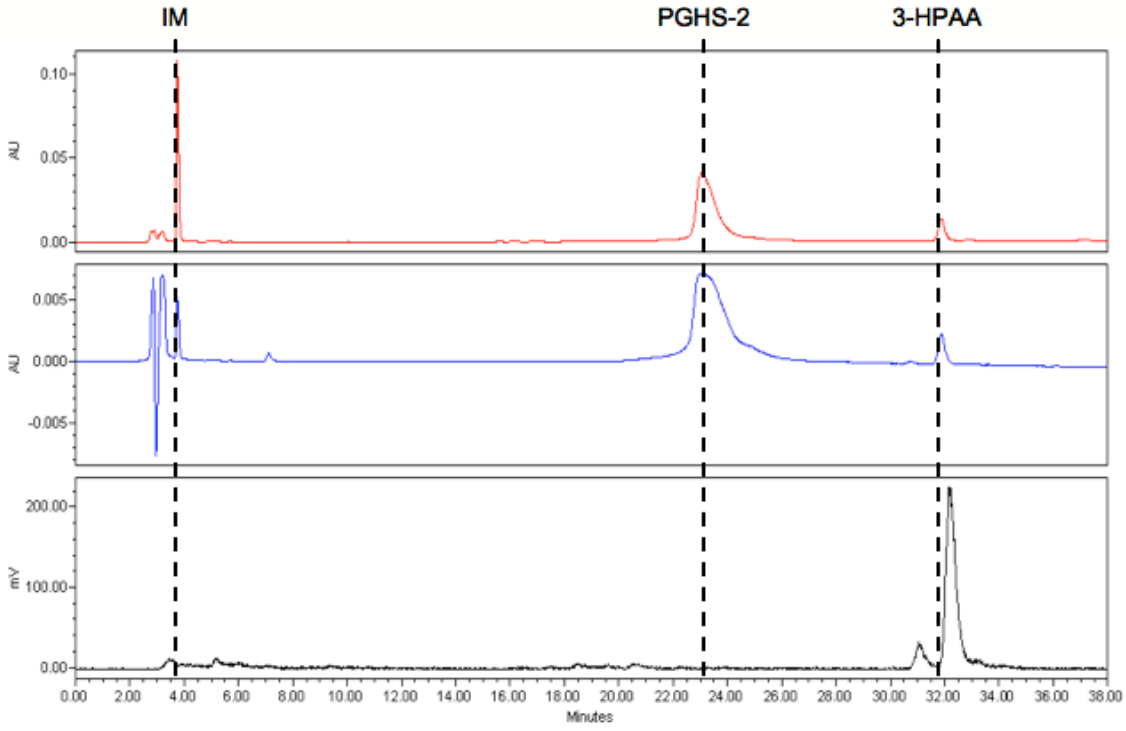
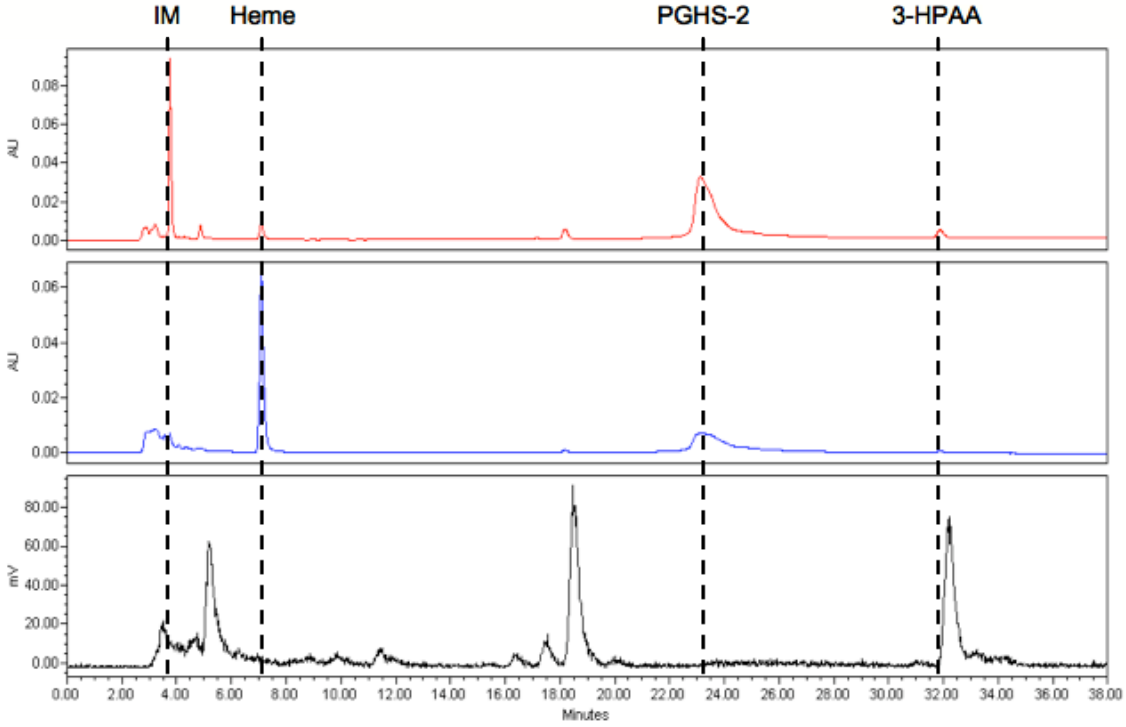


Figure S11. Irreversible inhibition of PGHS-2 by 3-HPAA. PGHS-2 was incubated with vehicle (*A*) or 10 μM 3-HPAA (*B*), and oxygen uptake curves were collected before (black curves) and after (red curves) ultrafiltration to remove excess inhibitor. Oxygen uptake was observed as a decrease in potential. PGHS-2 remained completely inactive even after removal of 3-HPAA by ultrafiltration.

A


IM
PGHS-2
3-HPAA
AU
AU
mV
Minutes

B


IM
Heme
PGHS-2
3-HPAA
AU
AU
mV
Minutes

C

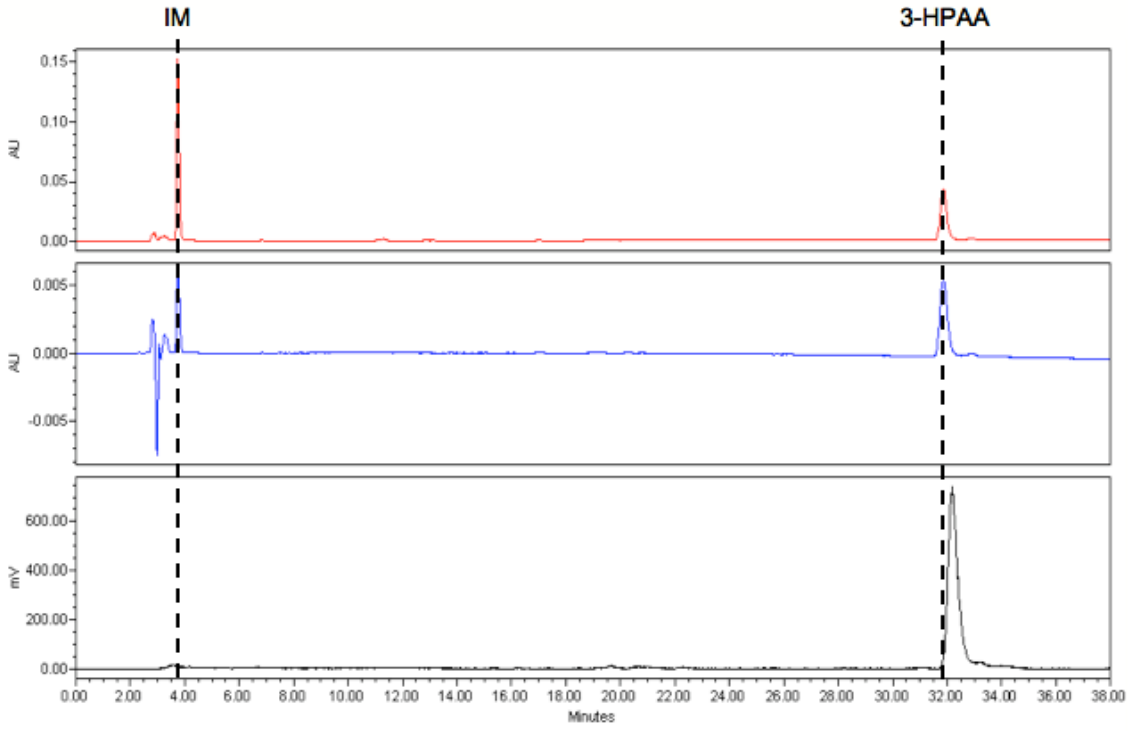
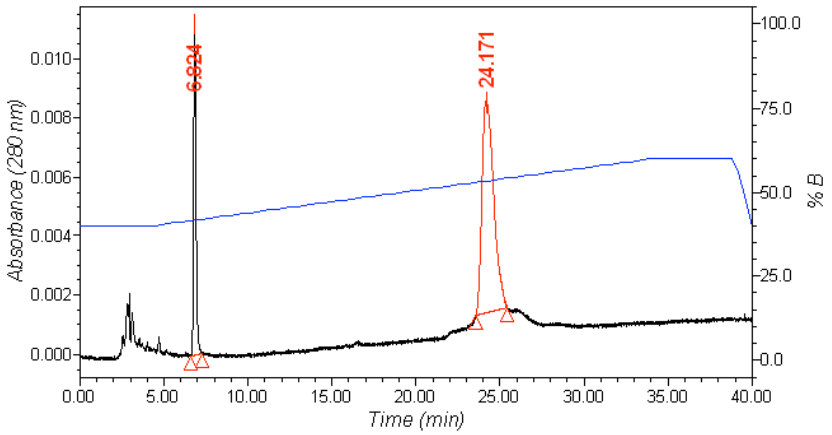
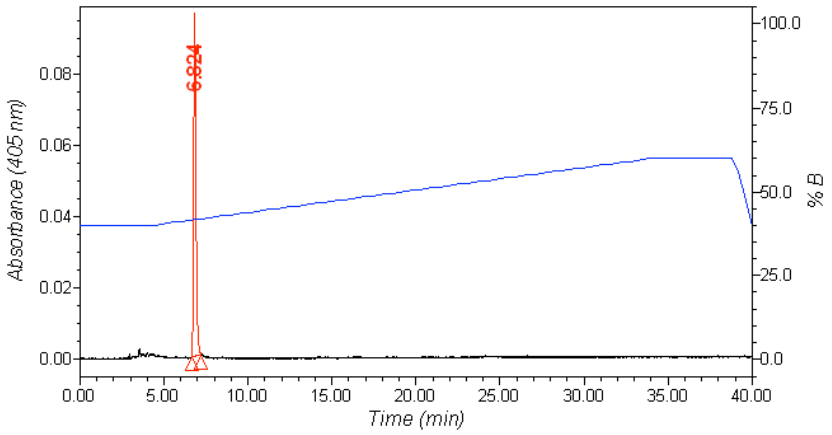
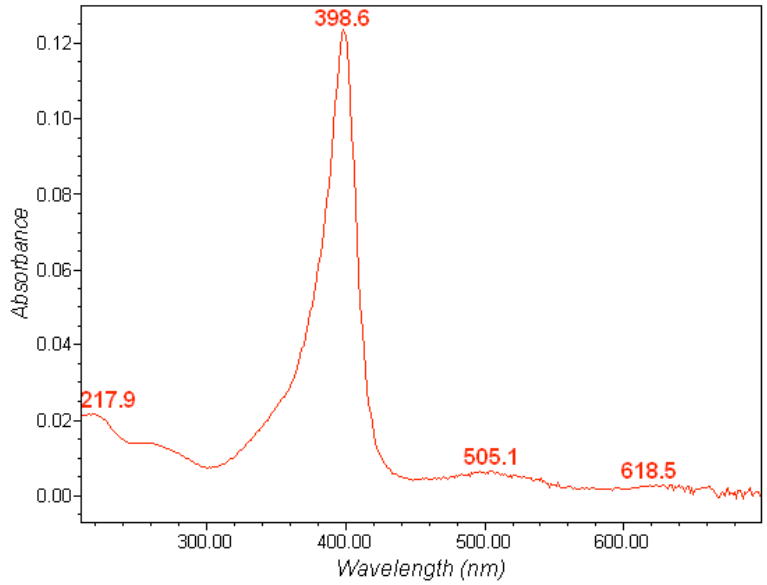


Figure S12. Assessment of covalent modification of PGHS-2 by 3-HPAA. *A,* Apo- or *B,* holo-PGHS-2 were incubated with two equivalents [^{14}C]-3-HPAA and analyzed on LC System C. Protein was detected by UV absorbance at 280 nm (red curve), heme at 405 nm (blue curve), and 3-HPAA by radiometric detection (black curve). Injection of [^{14}C]-3-HPAA is show in panel *C.* Elution of heme, PGHS-2 protein, and 3-HPAA are indicated by dashed marks. IM is the LC injection mark. Similar results were obtained when PGHS-2 was incubated with 5 equivalents 3-HPAA (data not shown).

A


6.824
24.171
Absorbance (280 nm)
% B
Time (min)


6.824
Absorbance (405 nm)
% B
Time (min)


398.6
217.9
505.1
618.5
Absorbance
Wavelength (nm)

B

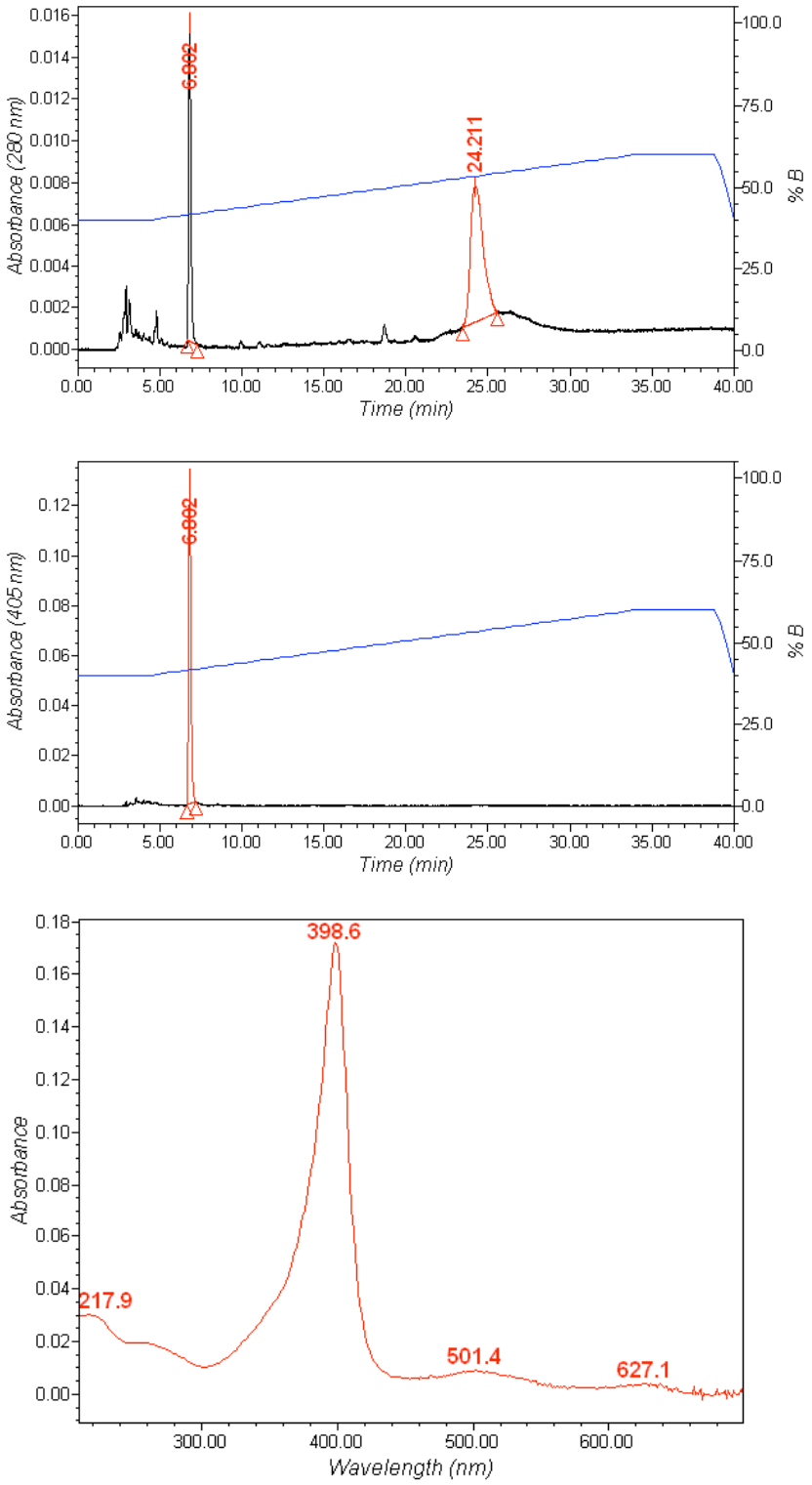


Figure S13. Assessment of PGHS-2 heme modification by 3-HPAA. Following incubation of holoPGHS-2 with *A,* vehicle, or *B,* 2 equivalents 3-HPAA, samples were analyzed by HPLC. Protein absorbance at 280 nm (top panel) and heme absorbance at 405 nm (middle panel) were monitored continuously. The

absorbance spectrum of the heme moiety was also characterized (bottom panel). No change in the retention time or absorbance spectrum of heme was observed. Similar results were obtained when PGHS-2 was incubated with 5 equivalents 3-HPAA (data not shown).

Table S1. Radiometric quantification of unreacted substrate and products generated by PGHS

	No enzyme		*PGHS-1*		*PGHS-2*	
	Region counts[b]	% ROI[c]	Region counts[b]	% ROI[c]	Region counts[b]	% ROI[c]
Unreacted 3-HPAA	8.3×10^5	96	9.8×10^5	84	4.9×10^5	87
Spot 2[a]	N.O.[d]	----	2.8×10^4	2.5	1.6×10^4	2.8
Spot 3[a]	N.O.[d]	----	3.1×10^4	2.7	1.1×10^4	2.0
Spot 4[a]	N.O.[d]	----	1.9×10^4	1.6	N.D.[e]	N.D.[e]

[a] Spots indicated by arrows in Fig. 8 are numbered sequentially from the uppermost spot. Spot 1 could not be quantified due to resolution of TLC. [b] Region counts, Total counts for the indicated spot. [c] % ROI, Region as a percentage of all counts above baseline. [d] N.O., Not observed. [e] N.D., Not determined (below limit of quantification)